PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P2725PCT-GN	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP 03/07534	International filing date *(day/month/year)* 13.06.2003	Priority date *(day/month/year)* 17.06.2002
International Patent Classification (IPC) or both national classification and IPC G06F19/00, G06F19/00		
Applicant BANYU PHARMACEUTICAL CO.,LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.09.2003	12.05.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Ulbrecht, M Telephone No. +49 89 2399-7710

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP 03/07534

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-38 as originally filed

Sequence listings part of the description, Pages

1-23 as originally filed

Claims, Numbers

1-23 as originally filed

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 17,23

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 17 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 23

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8,13,16,18-21
	No:	Claims	9-12,14,15,22
Inventive step (IS)	Yes:	Claims	8,21
	No:	Claims	1-7,9-16,18-20,22
Industrial applicability (IA)	Yes:	Claims	1-16,18-22
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re item III:

Claim 17 suggests an antibody specific for a variant ABCG2 polypeptide, however such an antibody is not supported by the description (Art. 6 PC) which does not disclose this feature of the invention in a way sufficiently clear and complete for it to be carried out by the skilled person (Art. 5 PCT). Consequently, no opinion will be given on claim 17 with respect to novelty, inventive step and industrial applicability.

Re item V:

Reference is made to the following documents:

D1: NAKATOMI KATSUMI ET AL: 'Mutation and quantitative analysis of breast cancer resistance protein (BCRP/MXR/ABCG2) gene in lung cancer cells and tumor tissues' PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 43, March 2002 (2002-03), page 778 93rd Annual Meeting of the American Association for Cancer Research;San Francisco, California, USA; April 06-10, 2002, March, 2002

D2: HONJO YASUMASA ET AL: 'Single-nucleotide polymorphism (SNP) analysis in the ABC half-transporter MXR/BCRP/ABCP1/ABCG2' PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 43, March 2002 (2002-03), pages 778-779, 93rd Annual Meeting of the American Association for Cancer Research;San Francisco, California, USA; April 06-10, 2002, March, 2002

D3: ALLEN JOHN D ET AL: 'A mutation hot spot in the Bcrp1 (Abcg2) multidrug transporter in mouse cell lines selected for doxorubicin resistance' CANCER RESEARCH, vol. 62, no. 8, 15 April 2002 (2002-04-15), pages 2294-2299,

D4: DOYLE L A ET AL: 'A MULTIDRUG RESISTANCE TRANSPORTER FROM HUMAN MCF-7 BREAST CANCER CELLS' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 95, December 1998 (1998-12), pages 15665-15670, cited in the application

D5: EP-A-1 323 735 (BANYU PHARMA CO LTD) 2 July 2003 (2003-07-02) which is the publication of the European patent application corresponding to WO 02 28894 A (BANYU PHARMA CO LTD ;HARA YOSHIKAZU (JP); KOMATANI HIDEYA (JP); KO) 11 April 2002 (2002-04-11) and whose content is therefore considered to be identical to the latter.

D6: DATABASE EMBL [Online] EBI; 21 October 2001 (2001-10-21) NCI-MGC:

'603176769F1 NIH_MGC_121 Homo sapiens cDNA clone IMAG:5240944 5"

D7: HONJO Y ET AL: 'Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells' CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 61, no. 18, 15 September 2001 (2001-09-15), pages 6635-6639

D8: IMAI YASUO ET AL: 'C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance' MOLECULAR CANCER THERAPEUTICS, vol. 1, no. 8, 17 June 2002 (2002-06-17), pages 611-616

D9: IIDA A ET AL: 'Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: ABCA4, ABCA7, ABCA8, ABCD1, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, ABCG2, ABCG4, ABCG5 and ABCG8' JAPANESE JOURNAL OF HUMAN GENETICS, TOKYO, JP, vol. 47, no. 6, 28 June 2002 (2002-06-28), pages 285-310

1.1 D6 discloses a polynucleotide consisting of more than 10 contiguous nucleotides of SEQ ID No. 1 and comprising an A at a position corresponding to position 34 of the ABCG2 mRNA (cf whole document). Thus, the subject-matter of claims 9-11 lacks novelty over D6 (Art. 33(2) PCT).

1.2 D3 discloses a pair of PCR primers which are capable of amplifying the complete coding sequence of the Abcg2 gene including positions 34, 376 and 421 (p. 2295, c. 1, §2). Hence, the subject-matter of claim 12 lacks novelty over D3 (Art. 33(2) PCT).

The subject-matter of claim 12 also lacks novelty over D4 (p. 15665, c. 2, §3) and D7 (Example 4) (Art. 33(2) PCT).

1.3 The polynucleotide of D6 (supra) is also considered to fall within the scope of claims 14 and 15, as it hybridises to the ABCG2 gene and is considered to be capable of detecting an A at position 34 of the ABCG2 mRNA, as well as of being used in one of the methods suggested by claim 15. Hence, D6 also takes away the novelty of claims 14 and 15 (Art. 33(2) PCT).

For the same considerations the polynucleotides (primers) disclosed in D3, D4 and D7 (supra) destroy the novelty of claims 14 and 15 (Art. 33(2) PCT).

1.4 The only technical feature of the kit according to claim 22 is the polynucleotide of any one of claims 9 to 11. Hence, D6 (supra) is also prejudicial to the novelty of claim 22 (Art. 33(2) PCT).

As an alternative embodiment of the kit according to claim 22 comprises only the pair of primers of claim 12, the subject-matter of claim 22 also lacks novelty over D3, D4 and D7 (supra).

1.5 The subject-matter of claims 1-8, 13, 16 and 18-21 is novel over the prior art which does not disclose the combination of features suggested by said claims (Art. 33(2) PCT).

2.1 D1 discloses that tumour cell lines bearing an alteration at amino acid position 141 of the ABCG2 transporter protein are resistance to SN-38 (cf whole document). As SN-38 is a drug transported by ABCG2 and drug resistance is a means of determining the drug transport capability of ABCG2 also used in the present application, the skilled person in applying routine skills would, based on the teaching of D1 determine an alteration at amino acid position 141 of ABCG2 expressed by a cell line in order to determine its resistance to SN-38 i.e. the transport capability of ABCG2 for said drug. Hence, the subject-matter of claim 1 does not involve an inventive step (Art. 33(3) PCT).

A similar conclusion would have to be drawn if the argumentation were started from D3. D3 discloses an enhanced efflux of daunorubicin and rhodamine 123 from mouse tumour cell lines caused by mutations in codon 482 of the mouse Abcg2 gene which lead to a substitution of R to either M or S in the Abcg2 transporter protein (p. 2296, c. 1, §2 - p. 2297, c. 1, §1; Fig. 3). Hence, D3 demonstrates the effect of a mutation at position 482 of Abcg2 on its transport capability for drugs. It would thus appear to fall within the scope of customary practice of the skilled person to determine a mutation (polymorphism) at said codon of the Abcg2 gene or at the corresponding position of the Abcg2 protein in order to predict the transport capability for said drugs. Consequently, the subject-matter of claim 1 appears to lack an inventive step (Art. 33(3) PCT).

As also D5 (Examples 10-14) and D7 (p. 6636, c. 2, §1 - p. 6639, c.1, §3; Fig. 1; Tab. 1) discloses an association of a mutation at said position of the human ABCG2 gene/protein with the transport capability for drugs, the same argumentation would apply, if it were started from the said documents (Art. 33(3)

PCT).

2.2 As elaborated under item 2.1 a method of determining the transport capability based on the ABCG2 polymorphism at amino acid position 141 is not considered to involve an inventive step in view of D1. In D1 tumour cell lines expressing said ABCG2 variant are used. Claim 19 differs from said closest prior art in that alternative cells are used. Substituting said cell lines by transformed cells expressing said ABCG2 variant requires nothing but routine experimentation and does not produce any unforeseeable effect and therefore does not involve an inventive activity (Art. 33(3) PCT).

2.3 For the same considerations as under 2.1, the application of the teachings of D1 to the assessment of SN-38 resistance by analysing biological samples from a subject as suggested by claim 20 does not establish an inventive step, in particular as D1 already teaches the ongoing analysis of lung tumour tissues and other human cancer tissues with respect to the ABCG2 polymorphism at amino acid position 141 (Art. 33(3) PCT).

2.4 D1 teaches a polymorphism of the ABCG2 polypeptide at position 141 which confers resistance to SN-38 (supra). D1 also teaches that said polymorphism is detected by direct sequencing of full length ABCG2 cDNA implying that the ABCG2 gene consisting of SEQ ID No. 1 was analysed at position 421. Hence, the additional feature of determining a polymorphism at position 421 as suggested by claim 2 is implicitly disclosed in D1 an does not establish an inventive step (Art. 33(3) PCT).

2.5 Based on the knowledge of the position of the ABCG2 polymorphism conferring SN-38 resistance (supra), the skilled person would in applying routine experimentation also arrive at the determination of the C421A polymorphism as suggested by claim 3 without the need of an inventive activity (Art. 33(3) PCT).

2.6 As D1 teaches the involvement of position 141 of the ABCG2 protein in SN-38 resistance, this feature proposed by claim 5 is already known from D1 and does not establish an inventive step (Art. 33(3) PCT).

2.7 For similar considerations as under 2.5 the subject-matter of claim 6 is considered not inventive (Art. 33(3) PCT).

2.8 The additional features suggested by claims 4 and 7 refer to routinely applied methodology which do not result in any unforeseeable technical effect and therefore do not establish an inventive step (Art. 33(3) PCT).

2.9 D2 discloses the G34A polymorphism of the ABCG2 gene as well as the corresponding V12M polymorphism of the ABCG2 protein (cf whole document). Starting from the teaching of D2 the isolation of a polypeptide according to claim 16 involves routine skills and does not result in any unforeseeable effect. Hence, claim 16 does not involve an inventive step (Art. 33(3) PCT).
As D1 teaches a polymorphism at 141 of ABCG2 (supra) the same considerations would also apply if the argumentation were started from D1 as the closest prior art (Art. 33(3) PCT).

2.10 Expression of the said protein in a transformed cell involves routine experimentation and does not produce any unexpected effect. Hence, claim 18 is considered not inventive (Art. 33(3) PCT).

2.11 The pairs of PCR primers according to claim 13 are considered to represent a mere selection of equally likely alternatives the skilled person would select without the need of an inventive skill (Art. 33(3) PCT).

2.12 The subject-matter of claim 8 differs from D1 representing the closest prior art in that the transport capability of a different compound is assessed. The technical problem lies in applying the teaching of D1 to a method of determining the transport capability of a different compound. None of the prior art documents suggests a compound according to claim 8 as being transported by ABCG2. Hence, the skilled person has no incentive to apply the teaching of D1 to a method of determining the transport capability of a compound according to claim 8. Claim 8 thus involves an inventive step (Art. 33(3) PCT).

2.13 The same considerations also apply to claim 21 (Art. 33(3) PCT).

3. Industrial applicability of claims 1-16, 18-22 is acknowledged (Art. 33(4) PCT).

4.1 Claims 12, 14 and 15 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("specifically hybridise ... amplify ... the amplified DNA fragment comprises ...", "specifically

hybridises ... capable of ...", "capable of using ...") which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should have been added.

4.2 The terms "drug transport capability" and "polymorphism" used in claim 1 are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claim unclear (Art. 6 PCT). For the purpose of examination the term "polymorphism" has been interpreted as also enclosing mutations and the term "drug transport capability" has been considered to refer inter alia to an altered resistance to drugs transported by ABCG2 as implicated by the analyses given in Example 3, namely Table 4 of the present application.

5. Certain published documents (Rule 70.10)
Should the priority of the present application not be valid, D8 and D9 would be relevant with respect to novelty and inventive step (Art. 33(2) and (3) PCT).